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Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

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REsCue trial: Randomized controlled clinical trial with extended-release calcifediol in symptomatic COVID-19 outpatients

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ABSTRACT

Objectives: This double-blind randomized controlled trial investigated raising serum 25-hydroxyvitamin D (25D) with extended-release calcifediol (ERC) on time to symptom resolution in patients with mild to moderate COVID-19.

Methods: COVID-19 outpatients received oral ERC (300 mcg on days 1–3 and 60 mcg on days 4–27) or placebo (NCT04551911). Symptoms were self-reported daily. Primary end points were raising 25D to ≥50 ng/mL and decreasing resolution time for five aggregated symptoms (three respiratory).

Results: In all, 171 patients were randomized, 160 treated and 134 (65 ERC, 69 placebo) retained. The average age was 43 y (range 18–71), 59% were women. The mean baseline 25D was 37 ± 1 (SE) ng/mL. In the full analysis set (FAS), 81% of patients in the ERC group achieved 25D levels of ≥50 ng/mL versus 15% in the placebo group ($P < 0.0001$). In the per-protocol (PP) population, mean 25D increased with ERC to 82 ± 4 (SE) ng/mL ($P < 0.0001$) by day 7; the placebo group trended lower. Symptom resolution time was unchanged in the FAS by ERC (hazard ratio [HR], 0.983; 95% confidence interval [CI], 0.695–1.390; $P = 0.922$). In the PP population, respiratory symptoms resolved 4 d faster when 25D was elevated above baseline level at both days 7 and 14 (median 6.5 versus 10.5 d; HR, 1.372; 95% CI, 0.945–1.991; $P = 0.0962$; Wilcoxon $P = 0.0386$). Symptoms resolved in both treatment groups to a similar extent by study end. Safety concerns including hypercalcemia were absent with ERC treatment.

Conclusion: ERC safely raised serum 25D to ≥50 ng/mL in outpatients with COVID-19, possibly accelerating resolution of respiratory symptoms and mitigating the risk for pneumonia. These findings warrant further study.

Introduction

It has been suggested that vitamin D repletion can reduce the risk for infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [1,2], mitigate severity of COVID-19 [3], and accelerate recovery [4]. Sufficient serum total 25-hydroxyvitamin D (25D) is postulated to potentiate COVID-19 vaccine effectiveness [5], boost innate and control adaptive immunity [6,7], and reduce

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post-infection cytokine storm [6] and lung injury [8]. A serum 25D level of 50 ng/mL is proposed as the theoretical threshold for zero mortality from COVID-19 based on regression analysis of decreasing deaths rates with increasing serum 25D levels [9].

The potential benefits of 25D repletion remain unsubstantiated in prospective randomized controlled trials (RCTs). In one RCT, administration of a single oral dose of 200,000 IU of cholecalciferol to patients with moderate to severe COVID-19 failed to shorten hospital stays despite raising mean serum 25D from a baseline of 21 to 44 ng/mL, diminishing support for vitamin D repletion [10]. In another RCT, a single oral dose of 100,000 IU of cholecalciferol at hospital admission did not improve COVID-19 disease outcomes [11].

The present study explored the benefit of raising serum 25D to ≥50 ng/mL, a level considered by some to be of concern [12], on time to resolution of symptoms in outpatients with COVID-19. The working hypothesis was that controlled, progressive elevation of 25D to this level with extended-release calcifediol (ERC) would safely boost the human innate immune response to SARS-CoV-2 and accelerate recovery via production of intracrine calcitriol in immune cells, such as macrophages, which co-express 25-hydroxyvitamin D-1α-hydroxylase (CYP27B1) and vitamin D receptor (VDR) in response to viral infection [13]. Selection of appropriate patient-reported outcomes as end points was hampered by the dearth of information regarding which COVID-19 symptoms respond to vitamin D treatment, leading to selection of a primary efficacy end point representing a "best guess" for finding a clinically meaningful vitamin D signal. To compensate, this proof-of-concept study explored a broad range of symptoms and used numerous prespecified and post hoc analyses to characterize potential signals.

Materials and methods

Study overview

This multicenter, randomized, double-blind, placebo-controlled phase 2 clinical trial titled REsCue (A Randomized, Double-Blind Placebo-Controlled Study to Evaluate the Safety and Efficacy of **R**ayaldee (calcifediol) **E**xtended-releas**e** **C**aps**u**-l**e**s to Treat Symptomatic Patients Infected with SARS-CoV-2), enrolled 171 symptomatic COVID-19 outpatients from 10 sites across the United States.

Randomization and masking

Patients were randomized 1:1 to 4 wk of treatment with ERC (30 mcg/capsule) or matching placebo and a 2-wk follow-up. The study was approved by Advarra (Columbia, MD) and overseen by an independent Data Safety Monitoring Board (DSMB) comprised of an expert in viral disease pathogenesis, a nephrologist, a biostatistician, a pulmonary medicine expert, and an endocrinologist experienced in clinical safety monitoring who reduced the maintenance dose to one capsule per day at day 21 for serum total 25D >100 ng/mL or serum total calcium (corrected for low albumin) >10.5 mg/dL. An interactive response technology provided treatment assignments using a computer-generated randomization code. Participants, study personnel, and the sponsor (and its designees, with the exception of the DSMB and three members of the data management team) were blinded to treatment assignments and serum vitamin D metabolite data until after database lock.

Procedures

ERC was chosen as the intervention because it has been previously shown to safely and effectively increase serum 25D to 50 to 100 ng/mL and suppress elevated intact parathyroid hormone (iPTH) when administered at 30 mcg/d and escalating, as needed, to 60 mcg/d in patients with stage 3 or 4 chronic kidney disease (CKD) [14]. Dosing was designed to increase 25D more quickly, but in a controlled, progressive manner, to the target range by day 7. The dosing protocol consisted of 300 mcg (10 capsules) on each of days 1, 2, and 3 followed by 60 mcg (2 capsules) on days 4 through 27, administered at bedtime after fasting for 3 h. Participants were instructed to remain fasting for 3 h after dosing. Thirty-four symptoms were self-reported daily using the FLU-PRO Plus questionnaire, an outcome tool validated for respiratory tract viral infections [15]. Symptoms were scored using multipoint scales ranging, most frequently, from 0 (*least severe*) to 4 (*most severe*). Blood samples and safety assessments were obtained at baseline and 7-d intervals.

Participants

Patients provided written informed consent and participated between November 2, 2020, and October 8, 2021 after testing positive for SARS-CoV-2 infection within the previous 3 d via reverse transcription polymerase chain reaction or substitutable FDA-authorized test. Patients were aged ≥18 y and had mild to moderate COVID-19, defined as the absence of clinical signs indicative of more severe disease such as oxygen saturation <94% or respiration rate >30 breaths per minute. All patients were required to have symptoms during screening with mean scores of ≥1.5 for each of the chest/respiratory and body/systemic domains of the questionnaire and instructed to forgo vitamin D supplements during the study. Patients were not excluded for serum 25D levels considered to be adequate (≥30 ng/mL) because the study sought to evaluate the pharmacologic effects of ERC administration rather than correction of vitamin D insufficiency (ie, restoring serum 25D to levels of ≥30 ng/mL). Patients were excluded if they were pregnant or breastfeeding; had recently taken systemic glucocorticoid medications; had primary hyperparathyroidism, kidney stones, hypercalciuria or hypercalcemia, cardiovascular disease, poorly controlled hypertension, arrhythmias, chronic granuloma-forming disease or chronic liver disease; history in the past 5 y of multiple myeloma or carcinoma of the breast, lung, or prostate; any condition that might significantly alter the metabolism of vitamin D; ongoing treatment with thiazide diuretics; history of hyperphosphatemia, hyperuricemia, or gout; estimated glomerular filtration rate (eGFR) <15 mL/min/1.73m^2; or serum calcium ≥9.8 mg/dL within the previous 3 mo.

Outcomes

One primary end point was attainment of the targeted serum 25D level by day 14. A second was time to resolution of five composite COVID-19 symptoms (trouble breathing, chest congestion, dry or hacking cough, body aches or pains, chills or shivering) which were part of the chest/respiratory and body/systemic domains of the questionnaire for which mean scores of ≥1.5 were required for enrollment. The composite respiratory symptoms (trouble breathing, chest congestion, dry or hacking cough) were considered indicative of pulmonary compromise. The other two composite symptoms (body aches or pains, chills or shivering) were considered indicative of systemic inflammatory responses to viral infection. An aggregate score for the primary end point related to symptoms was selected in view of the observed heterogeneity in COVID-19 symptom profiles. A score of ≤5, or a mean of ≤1 for each composite symptom, was deemed appropriate because the threshold of ≤1 described minor symptom severity (at worst "a little bit"), which seemed unlikely to presage a poor outcome. Resolution was defined as a reduction in the baseline symptom score (maximum of 4 for each included symptom) to or below ≤1 for a single symptom, ≤3 for three aggregated symptoms, and ≤5 for five aggregated symptoms for a minimum of 3 d consecutively. Secondary end points included time to resolution of each composite symptom and of aggregated symptoms as a function of serum 25D. Safety end points included adverse events detected by physical examinations, vital signs, electrocardiograms, hematology. and clinical chemistries. Special attention was given to changes in serum calcium and phosphorus, and eGFR, which presage potential hypercalcemia, hyperphosphatemia, and kidney damage. Exploratory end points included changes in serum 1,25-dihydroxyvitamin D (1,25D) and LL37 cathelicidin antimicrobial peptide, and plasma iPTH.

Laboratory procedures

BioReference Laboratories (Elmwood Park, NJ, USA) analyzed serum total 25D by liquid chromatography-mass spectrometry, plasma iPTH by electrochemiluminescence (Roche Elecsys, Greenbrook, NJ, USA) and serum total 1,25D by chemiluminescence (Liaison, DiaSorin, Cypress, CA, USA). Syneos Health (Quebec, Canada) analyzed serum LL37 by enzyme-linked immunosorbent assay (LSBio, Seattle, WA, USA). The circulating neutrophil-to-lymphocyte ratio was obtained from the complete blood cell count as routinely performed.

Statistical analysis

A sample size of 80 per group was planned and provided >80% power at one-sided α of 0.025 based on a log-rank test assuming 50% of placebo participants and 70% of ERC patients achieved resolution of symptoms. Results for the full analysis set (FAS) and per-protocol (PP) population are presented herein. The two primary end points were tested hierarchically to maintain an overall one-sided α level of 0.025. The first, attainment of serum 25D levels of ≥50 ng/mL, was assessed with a χ^2 statistic. The second, number of days to resolution of five aggregated symptoms, was analyzed with a Cox proportional hazards model with three covariates considered to have possible influence on treatment efficacy by log-rank test: baseline score for the five aggregated symptoms, baseline serum 25D, and

body weight. The Wilcoxon test was used instead of the log-rank test to compare Kaplan–Meier curves examining time to resolution of aggregated or individual symptoms because it is more suited to detecting differences in the earlier time points, given that the curves are expected to converge as patients normally improve by 28 d. Participants who did not achieve symptom resolution before ending participation in the study (2.9 to 6.7% depending on the symptoms being evaluated) were right censored after the last obtained data. Other efficacy end ppoints were analyzed by time point using appropriate *t* tests.

Results

Patients

In all, 241 patients provided written informed consent and were screened for eligibility (Fig. 1). Of these, 171 met selection criteria and were randomized to treatment. The most common reason for failing screening was insufficient severity of symptoms. Five individuals withdrew consent before dosing, and six failed to receive shipped study drug, leaving 160 participants (80 per treatment group) receiving at least one dose of study drug (the safety population). Eight patients from the ERC group and five from the placebo arm achieved symptom resolution before dosing and were excluded from analysis, leaving 147 participants (72 ERC and 75 placebo) in the FAS. Thirteen participants had major deviations from the protocol (e.g., <80% dosing compliance as documented in daily diaries) before resolution of symptoms and were excluded from the PP population, which consisted of 134 participants (65 ERC and 69 placebo). Baseline data for the PP population are shown in Table 1.

Outcomes

The first primary efficacy end point was attainment of serum 25D levels of ≥50 ng/mL. In the FAS, 81% of participants treated with ERC achieved this threshold versus 15% of those treated with placebo ($P < 0.0001$). Attainment of this end point was unaffected by body weight or body mass index. In the PP population, the corresponding percentages were 86% and 15% ($P < 0.0001$); mean serum 25D levels increased with ERC treatment to 82 ± 4 (SE) ng/mL ($P < 0.0001$) by day 7 and remained elevated for the duration of the study (Fig. 2A). In contrast, mean serum 25D trended lower with placebo treatment. Ten participants receiving ERC required dose reductions at day 21 due to 25D levels increasing

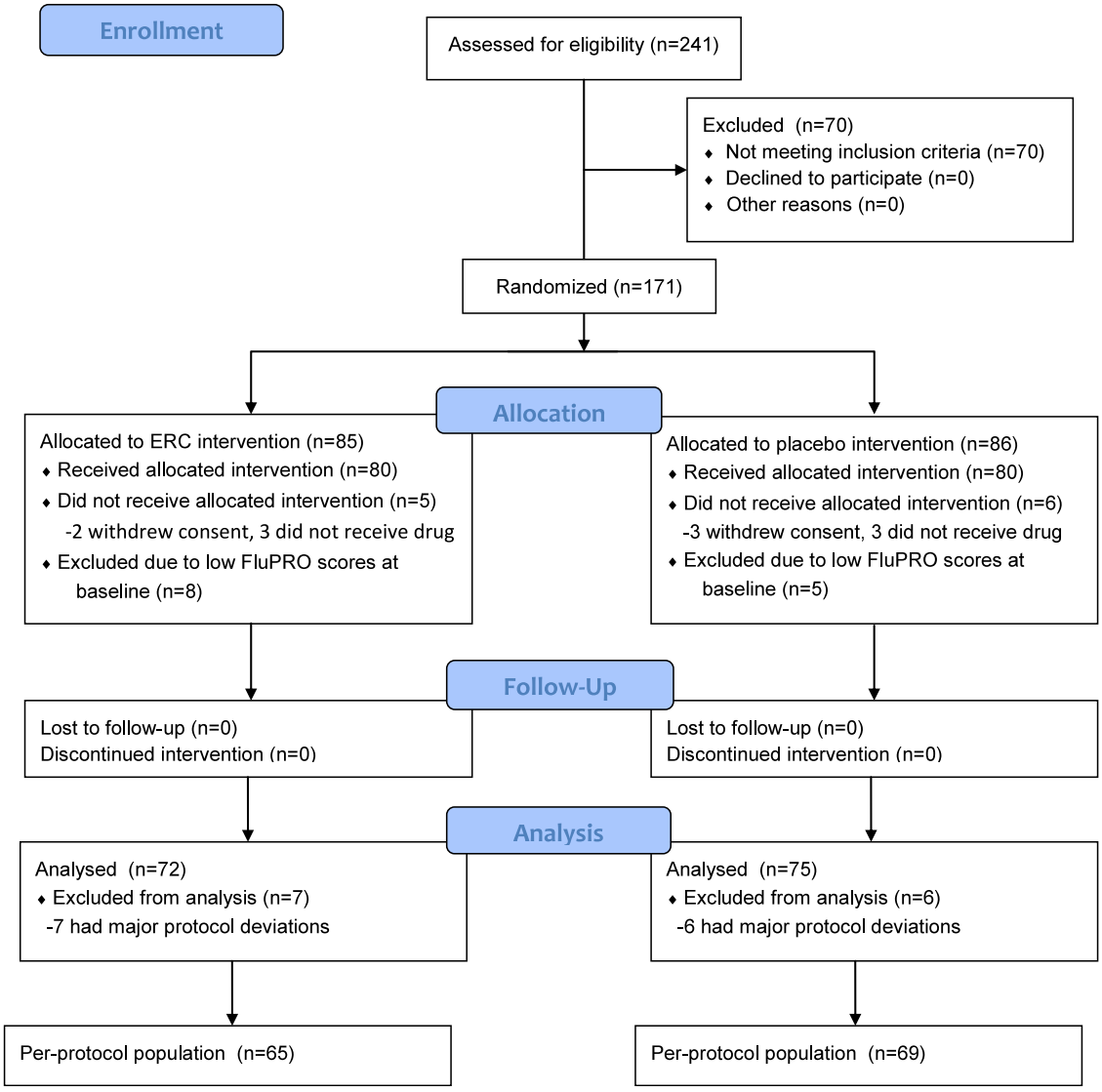

Fig. 1. Consort Diagram

Table 1
Baseline characteristics of per-protocol participants

	ERC (n = 65), n (%)	Placebo (n = 69), n (%)
Age, mean (SD), y	42.1 (14.1)	43.8 (14.3)
Sex, n (%)		
Men	27 (41.2)	28 (40.6)
Women	38 (58.8)	41 (59.4)
Race/Ethnicity, (%)		
Black	7 (10.8)	3 (4.3)
Other	0	1 (1.4)
White	58 (89.2)	65 (94.2)
Hispanic	52 (80)	55 (79.7)
Body mass index, mean (SD)	28.6 (5.5)	30 (5.9)
Acute COVID-19 symptoms, n (%)		
Trouble breathing	46 (70.8)	46 (66.7)
Chest congestion	49 (75.4)	59 (85.5)
Dry or hacking cough	57 (87.7)	57 (82.6)
Body aches and pains	63 (96.9)	68 (98.6)
Chills and shivering	52 (80)	52 (75.4)
Coexisting diseases, n (%)		
Hypertension	13 (20)	16 (23.2)
Type 2 diabetes mellitus	3 (4.6)	4 (5.8)
Asthma	1 (1.5)	2 (2.9)
Chronic kidney disease	0	4 (5.8)
Rheumatologic disease	3 (4.6)	2 (2.9)
Type 1 diabetes mellitus	1 (1.5)	0
COVID-19 vaccination, n (%)	3 (4.6)	1 (1.4)

ERC, extended-release calcifediol.

>100 ng/mL. ERC treatment produced similar mean increases in serum 25D in each baseline 25D category (Fig. 2B).

The second primary efficacy end point was the number of days to resolution for the five aggregated COVID-19 symptoms (three respiratory symptoms: trouble breathing, chest congestion, dry or hacking cough; two non-respiratory symptoms: body aches or pains, chills or shivering). In the FAS, resolution time with ERC treatment was unchanged, being 10.2 ± 8.22 d (mean ± SD) versus 10.8 ± 9.45 days with placebo treatment (hazard ratio [HR], 0.983 where a value >1.000 indicates earlier symptom resolution for the active group; 95% confidence interval [CI], 0.695–1.390; $P = 0.922$). Analysis of three covariates (baseline aggregate symptom score, baseline serum 25D concentration, and body weight) was negative. In the PP population, resolution time with ERC treatment remained unchanged, being 9.8 ± 8.15 d versus 10.8 ± 9.54 days with placebo treatment (median of 8 versus 6 d; HR, 1.114; 95% CI, 0.778–1.595; $P = 0.556$; Wilcoxon $P = 0.827$). Chest congestion (Fig. 3B) tended to resolve earlier in the PP population when serum 25D levels reached ≥50 ng/mL (8.3 ± 7.49 d and median of 5.5 d for the high 25D group versus 11.2 ± 8.91 and 8 d for the low 25D group; HR, 1.364; 95% CI, 0.881–2.110; $P = 0.164$; Wilcoxon $P = 0.0521$). When the three respiratory symptoms in the PP population were analyzed together post hoc, resolution occurred 4 d faster (Fig. 3C) when serum 25D was elevated above baseline at both days 7 and 14 (9.9 ± 9 d and median of 6.5 d in the increase group versus 12.3 ± 8.79 and 10.5 d for the no-increase group; HR, 1.372; 95% CI, 0.945–1.991; $P = 0.0962$; Wilcoxon $P = 0.0386$); chest congestion (Fig. 3D) resolved 2.5 d faster (8.6 ± 7.72 d and median of 6 d in the increase group versus 11.7 ± 9.02 and 8.5 d for the no-increase group; HR, 1.361; 95% CI, 0.922–2.009; $P = 0.121$; Wilcoxon $P = 0.0484$). At the end of the study, symptoms in both treatment groups had resolved to a similar extent.

The sample size of the study was too small to evaluate the effect of treatment on incidence of urgent care visits, oxygen saturation <94%, and hospitalizations. Table 2 summarizes the changes in laboratory measurements during treatment. Serum total 1,25D, serum LL37, and the neutrophil-to-lymphocyte ratio all trended upward with ERC treatment and downward with placebo, whereas serum calcium and phosphorus, plasma iPTH, and eGFR remained stable. Mean serum calcium in the ERC group was unaffected by baseline or post-treatment serum 25D levels. Episodes of hypercalcemia or hyperphosphatemia were not observed, even in patients whose serum 25D exceeded 100 ng/mL. Other safety end points showed no clinically meaningful changes with treatment.

Discussion

This RCT showed that ERC treatment was effective in increasing serum 25D levels to ≥50 ng/mL, which may have yielded significantly shorter resolution times for three aggregated respiratory symptoms (trouble breathing, chest congestion, and dry or hacking cough) commonly observed in patients with mild to moderate COVID-19. These findings suggest that raising serum 25D with ERC

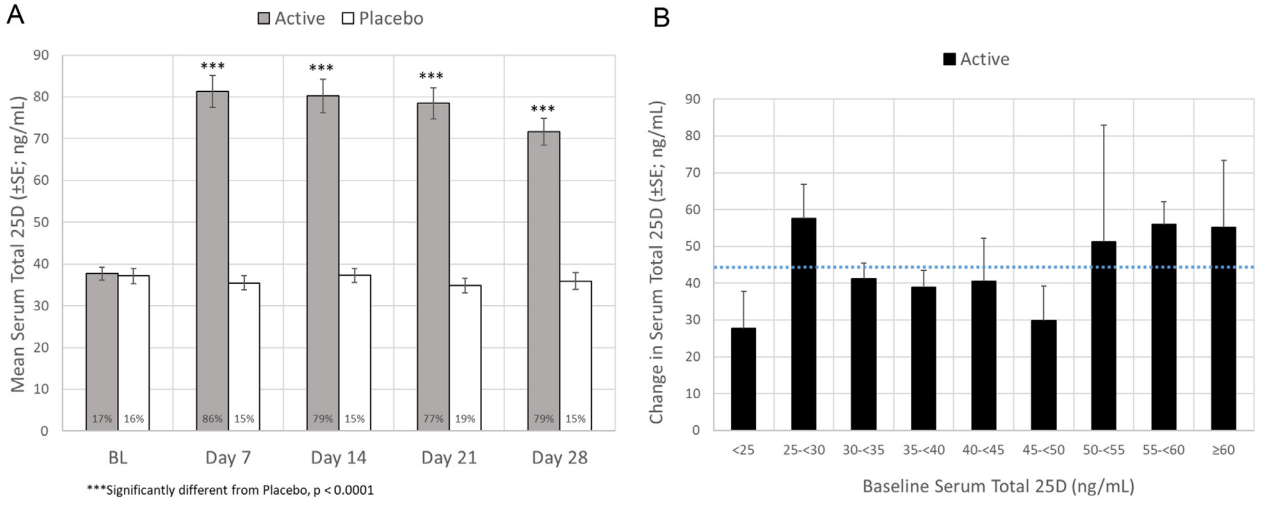

Fig. 2. (**A**) Mean (SE) Serum total 25-hydroxyvitamin D by study day and treatment group (per-protocol population). Percentages at the base of each bar indicate the proportion of participants achieving serum 25D levels of ≥50 ng/mL. *Significant differences between treatment groups ($P < 0.001$). (**B**) Mean (SE) increases in serum total 25-hydroxyvitamin D with ERC treatment by baseline serum 25-hydroxyvitamin D category (per-protocol population). The horizontal dotted line indicates the mean increase for all treated patients.

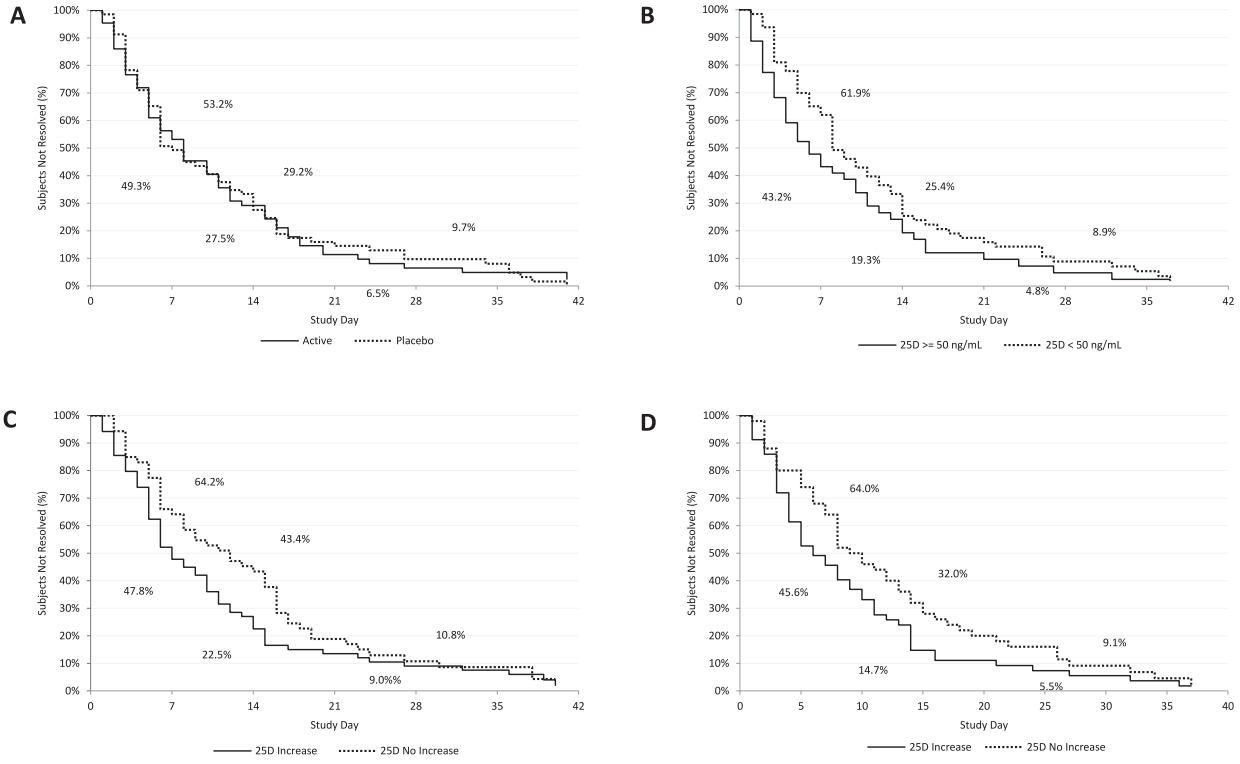

Fig. 3. (**A**) Kaplan–Meier curves displaying the time to resolution of five aggregated symptoms (trouble breathing, chest congestion, dry or hacking cough, body aches or pains, chills or shivering) by treatment group (per-protocol population). Active group: symptoms resolved in 61 patients and 4 were right-censored. Placebo group: symptoms resolved in 67 participants and 2 were right-censored. The difference between the plotted curves is not statistically significant: mean ± SD of 9.8 ± 8.15 d and median of 8 d for the active group vs 10.8 ± 9.54 and 6 d for the placebo group (HR, 1.114 where a value >1.000 indicates earlier symptom resolution for the active group; 95% CI, 0.778–1.595; $P = 0.556$; Wilcoxon $P = 0.827$). The probability of freedom from symptoms at days 7, 14, and 28 is 0.47, 0.71, and 0.94 for the active group and 0.47, 0.67, and 0.90 for the placebo group. The percentages of participants at risk in each group are shown at days 7, 14 ,and 28. (**B**): Kaplan–Meier Curves displaying the time to resolution of chest congestion in participants achieving at both days 7 and 14 serum total 25-hydroxyvitamin D levels of ≥50 ng/mL vs <50 ng/mL (per-protocol population). High 25D group: symptoms resolved in 42 participants and 2 were right-censored. Low 25D group: symptoms resolved in 61 participants and 2 were right-censored. The difference between the plotted curves is not statistically significant: mean ± SD of 8.3 ± 7.49 d and median of 5.5 d for the high 25D group vs 11.2 ± 8.91 and 8 d for the low 25D group (HR, 1.364 where a value >1.000 indicates earlier symptom resolution for the high 25D group; 95% CI, 0.881–2.110; $P = 0.164$; Wilcoxon $P = 0.0521$). The probability of freedom from symptoms at days 7, 14, and 28 is 0.57, 0.81, and 0.95 for the high 25D group, and 0.38, 0.75, and 0.91 for the low 25D group. Twenty-seven participants did not have chest congestion at treatment initiation (day 1) and were excluded from analysis. The percentages of participants at risk in each group are shown at days 7, 14, and 28. (**C**) Kaplan–Meier curves displaying the time to resolution of a composite of three respiratory symptoms (trouble breathing, chest congestion, dry or hacking cough) in participants achieving at both days 7 and 14 increases vs no increases in serum total 25-hydroxyvitamin D levels (per-protocol population). Increase group: symptoms resolved in 66 participants and 3 were right-censored. No-increase group: symptoms resolved in 50 participants and 3 were right-censored. Resolution occurred 4 d faster when serum 25D was elevated above baseline at both days 7 and 14 (mean ± SD of 9.9 ± 9 d and median of 6.5 d in the increase group vs 12.3 ± 8.79 and 10.5 d for the no-increase group; HR, 1.372 where a value >1.000 indicates earlier symptom resolution for the increase group; 95% CI, 0.945–1.991; $P = 0.0962$; Wilcoxon $P = 0.0386$). The probability of freedom from symptoms at days 7, 14, and 28 is 0.46, 0.85, and 0.94 for the increase group, and 0.42, 0.69, and 0.90 for the no-increase group. Sixteen participants did not have a total symptom score of >3 at treatment initiation (day 1) and were excluded from analysis. The percentages of participants at risk in each group are shown at days 7, 14, and 28. (**D**) Kaplan–Meier curves displaying the time to resolution of chest congestion in participants achieving at both days 7 and 14 increases vs no increases in serum total 25-hydroxyvitamin D levels (per-protocol population). Increase group: symptoms resolved in 55 participants and 2 were right-censored. No-increase group: symptoms resolved in 48 participants and 2 were right-censored. Chest congestion resolved 2.5 d faster when serum 25D was elevated above baseline at both days 7 and 14 (mean ± SD 8.6 ± 7.72 d and median of 6 d for the increase group vs 11.7 ± 9.02 and 8.5 d for the no-increase group; HR, 1.361 where a value >1.000 indicates earlier symptom resolution for the increase group; 95% CI, 0.922–2.009; $P = 0.121$; Wilcoxon $P = 0.0484$). The probability of freedom from symptoms at days 7, 14, and 28 was 0.54, 0.85, and 0.94 for the increase group, and 0.36, 0.68, and 0.90 for the no-increase group. Twenty-seven participants did not have chest congestion at treatment initiation (day 1) and were excluded from analysis. The percentages of participants at risk in each group are shown at days 7, 14, and 28.

may mitigate, without adverse effects, the risk for COVID-19 pneumonia in patients with mild to moderate COVID-19.

A study in Spain reported that administration of much higher bioavailable doses of immediate-release calcifediol (IRC) after hospitalization significantly reduced disease severity of COVID-19 [16]. In contrast, a single bolus dose (100,000 or 200,000 IU) of cholecalciferol failed to improve COVID-19 outcomes [10,11]. The effectiveness of bolus doses has been questioned because they upregulate cellular catabolism of calcitriol via 24-hydroxylation [17,18]. The present study focused on outpatients rather than on hospitalized patients because effective early intervention could prevent hospitalizations.

A possible mechanism [13] underlying ERC's potential effectiveness is induction of endogenous antimicrobial peptides (eg, LL37) that can boost immune responses to the virus. Serum LL37, which trended upward in this study, is the product of the cathelicidin antimicrobial peptide (*CAMP*) gene and is secreted from monocyte/macrophages, dendritic cells, and neutrophils in response to viral or bacterial infection. The innate immune response is initiated and perpetuated in antigen-presenting cells by pathogen-associated molecular patterns derived from the viral capsid and other unique signatures of the infecting virus interacting with pattern recognition receptors (e.g., Toll-like receptors [TLRs]). TLR activation leads to upregulation of expression of intracellular CYP27B1 and VDR;

Table 2
Changes in clinical chemistries from baseline to day 7 and day 14 by treatment group

Lab test, Mean (SD)	Treatment	Baseline (visit 1)	Day 7 (visit 2)	Day 14 (visit 3)
1,25-dihydroxyvitamin D (pg/mL)	ERC	71.1 (29.6)	78.4 (34.7)*	74.8 (23.8)†
	Placebo	74.1 (25)	66.6 (20.5)	59.4 (21.7)
25-hydroxyvitamin D (ng/mL)	ERC	37.7 (12.1)	81.8 (30.5)†	79.9 (32.6)†
	Placebo	37.1 (15.6)	34.8 (13.8)	36.4 (13.5)
Calcium corrected (mg/dL)	ERC	8.81 (0.37)	9.03 (0.38)	8.99 (0.34)
	Placebo	8.74 (0.37)	8.90 (0.33)	9.01 (0.35)
Glomerular filtration rate (mL/min/1.73m^2)	ERC	98 (17.07)	99.1 (16.4)	98.2 (14.4)
	Placebo	99.2 (19.4)	100.5 (19.9)	101.3 (18.6)
LL37 (ng/mL)	ERC	1.61 (1.41)	1.67 (1.43)	1.76 (1.68)
	Placebo	1.66 (1.63)	1.69 (1.43)	1.63 (1.32)
Neutrophil/lymphocyte ratio (%)	ERC	2.01 (1.24)	2.12 (1.05)	2.05 (1.00)
	Placebo	2.04 (1.07)	2.00 (1.06)	2.02 (0.73)
Parathyroid hormone, intact (pg/mL)	ERC	28.8 (14.7)	27.6 (15.9)	26.6 (11.5)
	Placebo	30.2 (13)	33.8 (15.1)	33.4 (14.6)
Phosphorus (mg/dL)	ERC	3.36 (0.49)	3.43 (0.61)	3.44 (0.54)
	Placebo	3.37 (0.65)	3.28 (0.52)	3.41 (0.61)

*Differences between treatment groups are significant: $P<0.05$.
†Differences between treatment groups are significant: $P<0.001$.

the former event upregulates the enzymatic conversion of the prohormone calcifediol to its active metabolite, calcitriol, which can then engage VDR in an intracrine mode and control *CAMP* gene expression [19]. Only in higher primates, including humans, is the *CAMP* gene regulated by calcitriol [20].

Serum 25D of about ≥50 ng/mL has been suggested to support intracellular generation of 1,25D, activation of the VDR, transactivation of the *CAMP* gene, and production and release of LL37 to combat SARS-CoV-2 proliferation in the host [19]. Serum levels of 1,25D and LL37 trended upward with ERC treatment, reflecting their elevated intercellular concentrations in the inflammatory microenvironment of the lung. The circulating neutrophil to-lymphocyte ratio, a purported biomarker of disease activity [21], increased in this study, but declined significantly with IRC in a previously reported RCT [22] conducted in hospitalized patients with COVID-19. In addition to this biomarker, no significant differences between IRC and placebo treatment were reported. Possible explanations for why the IRC findings diverged from the current findings include fewer participants (28 active, 34 placebo), more severe COVID-19, lack of a loading dose, more rapid release of calcifediol in the proximal bowel, and lower achieved serum 25D level (mean ± SD 42 ± 13.7 ng/mL). Release of calcifediol from ERC is gradual, continues over a period of 12 h (based on in vitro dissolution), and likely occurs primarily in the colon.

The current study differed from previous evaluations of vitamin D supplementation in patients with COVID-19 in two ways. First, it targeted a high serum 25D exposure (50−100 ng/mL) with downward dose adjustment, as needed. This approach was chosen in view of the reported inverse relationship between baseline 25D and observed increase with vitamin D supplementation [23] (a relationship not observed in this study), and the known adverse effect of adipose tissue on bioavailability of vitamin D supplements (cholecalciferol and ergocalciferol) that can be readily overcome with ERC [24]. Supplements are fat soluble, poorly absorbed in the intestine, accumulate preferentially in adipose tissue [25], and are poorly mobilized from adipose into circulation for hepatic activation [26]. They have multi-day delays in raising serum 25D [27], and prove to be unreliable in raising serum 25D in overweight or obese patients who are at elevated risk for COVID-19 [28,29]. Furthermore, hepatic vitamin D 25-hydroxylase activity is reduced in obesity, also blunting the intended elevation of serum 25D [30]. In contrast, calcifediol requires no hepatic activation, is more water soluble, and avidly binds to vitamin D binding proteein, reducing accumulation in adipose tissue and enabling ready availability to peripheral tissues, including virus-activated immune cells containing CYP27B1. Second, consistent with the excellent safety profile established for ERC in patients with stage 3 or 4 CKD [14], the slow-release formulation of ERC safely achieved high serum 25D exposures (50−100 ng/mL), which have been of concern for vitamin D supplements [12].

Strengths

The strengths of this study were the controlled design; targeting and safely achieving adequately high and sustained serum 25D levels of ≥50 ng/mL; broad examination of COVID-19 symptoms; and in-person monitoring of patient outcomes and safety.

Limitations

This study's inability to show more significant differences in time to resolution for respiratory symptoms may have been due to small sample size or the lack of any treatment effect. The fact that 17% of participants assigned to the placebo treatment had, unexpectedly, serum 25D levels >50 ng/mL at baseline; dosing noncompliance; and delays between onset of symptoms and diagnosis of COVID-19 and between diagnosis and initiation of treatment. The observed positive effect of serum 25D elevation on resolution of respiratory symptoms is based on a post hoc analysis and needs confirmation in a larger study.

Conclusions

ERC was effective in increasing serum total 25D to levels of ≥50 ng/mL in outpatients with mild to moderate COVID-19 and may have accelerated resolution of respiratory symptoms, suggesting mitigation of COVID-19 pneumonia risk. The positive findings from this RCT warrant confirmation in additional larger studies.

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